Committee:
Kotin, Chm.
Jacobson
Reimann

#253

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street, New York 17, N. Y.

Application for Research Grant

Date: October 6, 1959

1. Name of Investigator: Christopher M. Martin, M. D.#

2. Title: Assistant Professor of Medicine, and Director, Division of Infectious Diseases

3. Institution & Address: Seton Hall College of Medicine
24 Baldwin Avenue
Jersey City 4, New Jersey

4. Project or Subject: INTERACTIONS OF VIRUSES, CARCINOGENS, AND NUCLEIC ACIDS

Specific Aims: As a major corollary to virological studies described in USPHS Research Grant Application E-3257, "Transferrin-Virus-Metal-Carcinogen-Interactions,"* it is proposed to explore the several fundamental implications in the field of carcinogenesis arising from the "transferrin effect" on viral synthesis, as recently described by Martin and Jandl*. Specifically, it is proposed to study (1) the binding of carcinogens and carcinogen-metal complexes to nucleic acids; and (2) carcinogen-binding by viruses and its relation to induction of neoplasia.

5. Detailed Plan of Procedure:

A. Carcinogen-metal complexes and nucleic acids:

Selected mutagenic organic carcinogens of varying degrees of metal affinity will be studied by equilibrium-dialysis and differential centrifugation techniques, and their rate of binding to human DNA, RNA, cell nuclei, other cell fractions, and plasma proteins compared, in the presence and absence of chelating agents. Binding affinities in the presence of various trace metals will be studied, particularly those metals shown to be both nucleic-acid associated and carcinogenic (nickel, chromium).

B. Carcinogen-binding by Viruses:

The uptake of fluorescent organic carcinogens by viruses and by viral nucleic acid will be studied by the techniques noted above and a

Copies of Curriculum Vitae attached.
* Copies attached.

variety of tissue culture and animal systems explored with a view to demonstrating the possible role of viruses or viral nucleic acids as vectors for the delivery of carcinogens to susceptible intracellular loci. Depending upon the results of *in vitro* affinity studies, various virus-carcinogen pairs will be administered to appropriate animal hosts, and the tumor-producing efficiency of virus plus carcinogen separately, virus-carcinogen complex, virus alone, and carcinogen alone will be compared with suitable controls. Particular attention will be given to respiratory tract viruses (influenza, adenoviruses, hemadsorption viruses). Tissue culture systems will include human embryonic kidney, lung, and tracheo-bronchial epithelium. For the present, animal studies will be limited to mice and hamsters.

C. Sources of tissues:

Human embryonic tissues derived from spontaneously aborted fetuses up to 4 months gestational age, suitable both for tissue culture and for cell fractionation studies, will be available through the Obstetrics and Gynecology Department of the Margaret Hague Maternity Hospital at the Jersey City Medical Center.

D. Metal determination techniques:

See enclosed USPHS E-3257, page 5.

6. Budget Plan:

	1st Year	2nd Year
Salaries	$6,000	$7,000
Expendable Supplies	3,000	2,000
Permanent Equipment	5,000	1,000
Overhead 15% (1)	1,350	1,350
Other	-	-
Total	$15,350	$11,350

(1) Excluding permanent equipment item.

7. Anticipated Duration of Work:

2 years: January 1, 1960 - December 31, 1961

8. Facilities and Staff Available:

January 1, 1960 - May 1, 1960: Temporary laboratory in Department of Microbiology, Seton Hall College of Medicine, fully equipped to conduct virus research (freezer, refrigerator, incubator, storage facilities; spacious animal quarters shared by pre-clinical departments; adequate space for ultracentrifugation studies with Spinco Model L and for equilibrium dialysis studies; inadequate space for metals analysis studies). Permanent laboratory facilities (estimated cost $39,000) under construction at this time.

June 1, 1960 - December 31, 1961: Permanent facilities in the Jersey City Medical Center completed, consisting of 2 virology-bacteriology laboratories; 1 chemistry laboratory with 12-foot fume hood, extensive bench space, centrifuges, spectrophotometer, automatic precision balance, plus equipment applied for in USPHS E3257.

Personnel: January 1, 1959 - June 30, 1959: Investigator, 2 technicians, secretary, glassware diener.

July 1, 1959 - December 31, 1960: Investigator; 2 Research Fellows (Dr. Clyde Wu - present address: Boston City Hospital, Boston 18, Mass.; Dr. Regina McCormack - present address: University of Virginia Hospital, Charlottesville, Va.); 2 technicians, secretary, glassware diener. (Bulk of support for this group to come from USPHS E3257 and 2 USPHS post-doctoral fellowships.)

9. Additional Requirements: (Explanation of Budget Plan)

1) Personnel - $13,000 - major portion of salaries of 1 technician and secretary for 2 years.

2) Permanent Equipment: $5,000 - including portion ($3,000) of cost of Spinco Model L Ultracentrifuge and of Zeiss Spectrophotometer ($2,000).

10. Additional Information (including relation of work to other projects and other sources of supply):

A. Background of Studies:

The proposed studies of virus-carcinogen-nucleic acid interactions represent a logical extension of increasing evidence that (1) Viral nucleic acid is an extremely reactive substance, chemically; and (2) Viral infections can be viewed as repeated assaults on the basic cellular genetic apparatus by mutagenic substances.

The long-term implications of such repeated interactions of cell and virus nucleic acid, interactions which occur primarily in youth - the period of rapid cellular growth - have barely been studied.

B. Previous Work Done on This Project:

See USPHS E3257, pages 8-9.

C. Results Obtained by Others:

The large body of evidence that viral nucleic acid is the component which endows viruses with cell- (and nuclei-) invasive properties has been reviewed by Colter (1). The most striking recent demonstration that the nucleic acid of common human enteroviruses possesses such properties, which the protein components merely modify, has been reported by Schaffer and Mattern (2).

Following the fundamental studies of Zinder and Lederberg (3) on transduction in Salmonella, many workers, particularly in the field of bacteriophage, have demonstrated the mutagenic properties of viral nucleic acid; these data have been reviewed by Luria (4). The indirect evidence gathered by Lederberg (5) supporting the concept of such phenomena occurring in animal cells has been extended by Benzer (6) and received striking support from the studies of Ebert (7) who has demonstrated the ability of Rous sarcoma virus to act as a vector in carrying exogenous, genetically active tissue DNA into chick embryo cells.

The intimate association of trace metals with nucleic acids has recently been quantitated and partially defined by Wacker and Vallee (8); studies by several investigators on the effect of trace metals and of chelating agents on the integrity of nucleic acid chains and protein-nucleic acid bonds in nucleoproteins have been summarized by Kirby (9).

The mutagenic properties of chemical carcinogens, first demonstrated by Tatum in Neurospora (10), have since been amply confirmed; progress in relating carcinogen action to alterations in specific gene loci has been reviewed and extended by Barratt and Tatum (11).

Scattered evidence for metal-carcinogen interaction has been summarized by Furst (12). Although suggestive evidence of interaction and synergism between viruses and chemical carcinogens was adduced as early as 1944 by Rous and Friedewald (13) and in 1952 by Duran-Reynals (14), there have been no published systematic studies of such interactions in biochemical terms. Stanley (15) has speculated that mutagenic carcinogens might induce neoplasia-stimulating properties in otherwise innocuous viruses or proviruses present in cells in the carrier state.

REFERENCES

1. Colter, J.S. Nucleic acid as the carrier of viral activity. Chapter in Progress in Medical Virology, Vol. I, Ed. by Berger, E. and Melnick, J.L. Hafner, New York, 1958, pp. 1-35.

2. Schaffer, F.L. and Mattern, C.F.T. Infectivity and physiochemical studies on RNA preparations from highly purified poliomyelitis and Coxsackie viruses. Fed. Proc. 18: 317, 1959.

3. Zinder, N.D., and Lederberg, J. Genetic exchange in Salmonella. J. Bact. 64: 679, 1952.

4. Luria, S.E. Mutations of viruses in relation to normal and abnormal cell functions. Ann. N.Y. Acad. Sci. 71: 1085-1091, 1958.

5. Lederberg, J. Cell genetics and hereditary symbiosis. Physiol. Rev. 32: 403-430, 1952.

6. Benzer, S. The Elementary Units of Heredity. Chapter in The Chemical Basis of Heredity, Ed. McElroy, W.D. and Glass, B., Johns Hopkins Univ. Press, 1957, pp. 70-93.

7. Ebert, J.D. (Carnegie Institute, Baltimore) Studies on Rous sarcoma virus. Paper presented at seminar, Harvard Medical School, Dept. of Bacteriology and Immunology, February, 1959.

8. Wacker, W.E.C., and Vallee, B.L. Chromium, manganese, nickel, and other metals in RNA. Fed. Proc. 18: 345, 1959.

9. Kirby, K.S. A new method for the isolation of desoxyribonucleic acids: Evidence on the nature of the bonds between desoxyribonucleic acid and protein. Biochem. J. 66: 495, 1957.

10. Tatum, E.L. Chemically induced mutations and their bearing on carcinogenesis. Ann. N.Y. Acad. Sci. 49: 87-97, 1947.

11. Barratt, R.W. and Tatum, E.L. Carcinogenic mutagens. Ann. N.Y. Acad. Sci. 71: 1072-1084, 1958.

12. Furst, A. Chelation and cancer - a speculative review. Symposium on Metal-Binding in Medicine. Philadelphia, May 6-8, 1959. To be published (J.B. Lippincott).

13. Rous, P. and Friedewald, W.F. The effect of chemical carcinogens on virus-induced rabbit papillomas. J. Exp. Med. 79: 511, 1944.

14. Duran-Reynals, F. Studies on the combined effects of fowl pox virus and methylcholanthrene in chickens. Ann. N.Y. Acad. Sci. 54: 977, 1952.

15. Stanley, W.M. Relationships, established and prospective, between viruses and cancer. Ann. N.Y. Acad. Sci. 71: 1100-1113, 1958.

D. Discussion:
Significance of this Research:

Persistent failure by many investigators in many systems to isolate a human "cancer virus" necessarily raises the question as to whether such a virus exists at all. On the other hand, there is abundant evidence, both in animals and in man, that a large number of chemical carcinogens will induce neoplasia. At the same time, however, efforts to demonstrate the etiologic role of proven environmental carcinogens in induction of neoplasia in man are in virtually all instances (G.I. tract, lung, liver) open to the objection that while experimentally exceedingly large doses of carcinogens are required to induce cancers, each of the suspect environments (artificial food dyes, industrial fumes, gasoline fumes, cigarette smoke, etc.) contains exceedingly minute quantities of carcinogen.

The present study proposes to explore a relatively plausible set of conditions, susceptible to controlled experimental analysis, which might square the negative virologic data and the discrepant carcinogen data; i.e., that minute amounts of carcinogens - not ordinarily of mutagenic significance - may induce neoplasia if bound to any of several common viruses - not ordinarily carcinogenic - and thereby delivered to the chromosomes of cells the virus invades but does not destroy.

The evidence that such interactions can occur is summarized above. If true and widespread, these interactions would carry the implication that neoplasia so induced is preventable (through virus vaccines) and that minute quantities of environmental carcinogens are relatively tolerable.

Signature /s./ Christopher M. Martin, M.D.
Director of Project

/s./ Charles L. Brown, M.D.

Dean
Seton Hall College of Medicine